Effect of Different Extraction Conditions on the Yield of Pectin Extracted from Bilimbi (*Averrhoa bilimbi*)

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ABSTRACT

Pectin is a naturally occurring biopolymer that is widely used in the food, pharmaceutical industry and biotechnology. Pectin from bilimbi (*Averrhoa bilimbi*) was extracted at different conditions. Pectin extraction was done considering varied time (30 min, 60 min and 120 mins), pH (1.5, 2.0 and 2.5) at and temperature (70°C, 80°C and 90°C). The highest yield of crude pectin was observed after 120 minutes heating 90°C and pH 2.0. Statistical analysis indicated that variations of temperature, pH and time had significant effects on the yield of pectin.

KEYWORDS: *Bilimbi, Extraction, Optimization, Pectin, Yield*

1. INTRODUCTION

Bilimbi (*Averrhoa bilimbi*) is a fruit-bearing tree from the genus *averrhoa* plant family. It is known as "cucumber tree" and "tree sorrel" but it is more widely known as bilimbi. It is a widely known fruit in different parts of Asia. In Malaysia, bilimbi is known as *belimbing asam, belimbing buloh, b'ling,* or *billing-billing.* Indonesians call it *belimbing besu, balimbing, blimbing,* or *blimbing wuluh;* in Thailand, it is *taling pling,* or *kaling pring;* in Vietnam, it is known as *khe tay.* Filipinos generally call it *kamias* or *kalamyas* [1][2][3].

Bilimbi is a small tree that grows from 5 to 10 meters high. The fruit is crisp when unripe but turns from bright-green to yellowish-green, ivory or nearly white when ripe and falls to the ground. The flesh is green, jelly-like, juicy, sour and extremely acidic due to high oxalic acid content [2][3][4]. It is commonly used as souring agent in most Filipino dishes and is used to make pickles, curries, chutney, and preserves. It is also made as refreshment drink and marketed similar to lemonade. Due to its high acidity and high oxalic acid content, the fruit is used for bleaching stains in clothes, for removing rust stains and cleaning knife blades. The leaves, fruits and other parts of the tree are also used in traditional medicine for the treatment of coughs, itches, skin swellings, and rheumatism [2][3][4][5].

Unlike other tropical fruits, bilimbi is one of the underutilized fruits in the Philippines and in Asia. It has not received much attention as compared to other commercial fruits like guava, papaya and pineapple. This could be due to its very sour taste and high acidity, lack of popularity among local communities, lack of information on nutritional compositions and lack of promotional campaigns for these fruits [6]. One way to increase its market value is to process it and turn it into a functional food ingredient. Pectin is a high value functional food ingredient widely used as gelling agent and as stabilizer [7]. It is interesting to note that the utilization of bilimbi as a source of pectin has not been reported so far. Limited information concerning pectin concentration in bilimbi is limited. On the other hand, star fruit (*Averrhoa carambola*), a close relative of bilimbi, has a pectin content of 0.04-0.60 % [8][9].

The Philippines depends largely on imported pectin due to the absence of a viable technology to produce it locally. In 2011, the Philippines imported a total of 94,848.93 kg of pectin from various origins with total custom value of US$ 52,383,487 or Php 2.2 billion. The cost of imported pectin is estimated at around Php 27,000/kg [10][11]. These were mainly used by the food processing, cosmetics and pharmaceutical industries as thickening, gelling and stabilizing agents.

In plant cells, pectin consists of a complex set of polysaccharides that are present in most primary

cell walls and particularly abundant in the non-woody parts of nearly all terrestrial plants [12]. Pectin is primarily a polymer of D-galacturonic acid. The principal and key feature of all pectin molecules is a linear chain of (1-4)-linked-a-D-galactopyranosyluronic acid units, making it an a-D-galacturonan [a poly (a-D-galactopyranosyluronic acid) or an a-D-galacturonoglycan] [13].

Numerous studies on pectin extracted from different sources have been reported. The physicochemical properties of pectin depend mainly on the plant source and conditions selected for its isolation and purification. Extraction is therefore an important step in the isolation and recovery of pectin [10][12][14][15][16].

The most commonly used technique for the extraction of pectin is the direct boiling or water based extraction method [10] [12] [14] [15] [16] [17] [18] [19] [20]. However, this method, due to long extraction time, may result in the degradation of the extracted pectin [12] [14] [20]. It is therefore essential to determine the best method for pectin extraction using the best condition to yield the highest quality. Extraction temperature, pH and extraction time are considered to be the most important factors in pectin extraction because they have shown significant effects on different pectin characteristics such as molecular weight, degree of esterification and yield [7] [21].

Utilization of pectin from bilimbi may help increase its market or commercial value; also wastage of the fruit may be avoided since the utilization of the fruit may be avoid due to increased. Farmers may utilize its full potential and hence improve their economic returns. Moreover, it can help reduce the country's dependence on imported pectin and may open many opportunities for farmers or food processors to produce different products from bilimbi or other underutilized tropical fruits.

Objectives of the Study

This study aimed to (1) extract pectin from bilimbi in a hydrochloric acid extraction process; (2) investigate the effects of three extraction parameters (pH, temperature and time) on the pectin yield; and (3) determine the best condition that will yield the highest amount of pectin from bilimbi.

2. MATERIALS AND METHODS

Materials

Matured bilimbi fruits were collected in Batangas City, Philippines. The fruits were washed thrice with tap water followed by distilled water to remove the dirt and other plant debris. The fruits were cut into half and oven dried (Memmert, Germany) at 55°C until a constant weight was is achieved. The dried fruits were ground to uniform size using a food processor at high speed and packed in a sealed polyethylene bags for the extraction of pectin.

All chemicals and solvents used were of analytical reagent grade.

Pectin Extraction Process

Pectin was extracted based on the method discussed by [10]. The ground bilimbi was subjected to hydrolysis by treating the bilimbi powder with distilled water acidified with 0.1 N HCl (Merck) at different pH levels (1.5, 2.0 and 2.5), extraction temperatures (70°C, 80°C and 90°C) and heating times (30, 60 and 120 minutes) to establish the extraction condition that would give the highest pectin yield. The resulting extract was cooled and filtered to remove solids/insoluble residues to obtain the pectic liquor. Extraction was done twice. The pectic liquor was precipitated with 95 percent ethanol (1:2 v/v). The mixture was left overnight to allow for the complete precipitation of pectin. The pectin was recovered from the liquor by passing through silk cloth. The pectin obtained was purified with successive washings with ethanol and a final rinse was done with fresh 95 percent ethanol. The fibrous pectin was oven dried at a temperature not more than 60°C, pulverized to a fine powder, sieved and stored in clean, dry air tight container. Percent recovery of pectin from bilimbi was determined by computing the ratio of pectin produced over the amount of the raw materials utilized. The pectin yield is

$$\%Yield = \frac{mass\ (g)\ of\ pectin\ produced}{mass\ (g)\ of\ \ bilimbi\ used\ for\ extraction}\ x\ 100 \quad (1)$$

3. RESULTS AND DISCUSSION

Percent Yield of Pectin from Bilimbi at Different Extraction Conditions

Table 1 shows the pectin yields from bilimbi at different extraction conditions.

Table 1
Percent Yield of Pectin* from Bilimbi at Different Extraction Conditions

	pH 1.5			pH 2.0			pH 2.5		
	30	60	120	30	60	120	30	60	120
70°C	4.75± 0.02	6.81± 0.04	9.19± 0.04	5.67± 0.05	6.66± 0.02	7.46± 0.05	6.38± 0.07	7.38± 0.05	8.19± 0.05
80°C	5.54± 0.03	7.37± 0.035	10.36± 0.06	7.97± 0.04	9.19± 0.03	11.98± 0.05	6.76± 0.1	8.03± 0.07	9.21± 0.11
90°C	5.99± 0.03	8.63± 0.04	9.57± 0.05	8.78± 0.07	11.57± 0.05	14.35± 0.04	7.31± 0.1	9.98± 0.13	11.03± 0.20

*Note: *mean values*

The yield of acid-extracted pectin ranged from 4.75% to 14.35% of the dry weight of the fruit. The highest yield was obtained when dried bilimbi fruit was extracted with acidified water at pH 2 for 120 minutes at 90°C.

The yield of pectin from bilimbi was compared to different raw materials, shown in Table 2.

Table 2
Pectin Yields from Different Raw Materials

No	Raw materials	Pectin Yield, %	Reference
1	Passion Fruit Peels	14.60	Liew, et al. [14]
2	Apple Pomace	8.61	Miceli-Garcia [7]
3	Chickpeas	8	Urias-Orona, et al., [22]
4	Cocoa Husk	7.62	Chan and Choo [16]
5	Strawberry	1.67	Kumar et al. [17]
6	Apricot	0.78	Aprajita et al. [23]
7	Gooseberry	19.68	Kumar et al. [17]
8	Saba Banana	17.05	Castillo-Israel [19]
9	Sugar Beet Pulp	16.2	Yapo et al. [24]
10	Water Melon Rind	20.7	Campbell [25]
11	Mango Peel	21.65	Gragasin et al. [10]
12	Bilimbi	14.35%	Present study

extraction of pectin Hydrochloric acid was used in the due to its high ionic strength which is higher than weak acids such as citric acid which are employed in some pectin extraction studies [14] [16] [17] [26] [27] [28]. It Also, higher ionic strength acids have a higher capability to precipitate pectin due to their higher affinity for cations such as Ca^{+2} which stabilizes the pectin molecule [19].

Also, as shown in table 1, the yield of pectin increases as extraction time and temperature increased. The pectin yield increased as the reaction progresses because longer extraction time provides more reaction time opportunity. On the other hand, pectin yield increased with increase in acidity from pH 2.5 to pH 2 and decreased at pH 1.5.

Effect of Extraction Time on Pectin Yield

Pectin yield generally increases as extraction time increases. Highest yield was achieved at 120 minutes of extraction while the lowest yield was observed at extraction time of 30 minutes. Statistical analysis revealed that increasing the extraction time from 30 minutes to 120 minutes, at constant pH and temperature, significantly increased the yield ($p<0.05$). At constant pH and temperature, pectin yield is seen to be higher at longer extraction time. Similar observations were noted in these studies of: Miceli-Garcia [7] Gragasin [10] Liew [14] Chan[16] Girma [18] Castillo-Israel [19] Yapo [24] and Vriesmann [27]. However, it is revealed that extraction at higher temperature at was extraction time would lead to decomposition of pectin. Since pectin is composed of α-(1-4) linked units of galacturonic acid or methyl ester, it can undergo hydrolysis reaction at the right conditions [29]. The degradation is mainly due to the depolymerization mechanism of galacturonan chain of pectin which is known as beta-elimination. Thus, the pectin cannot be recovered by precipitation with alcohol [30].

Effect of Extraction PH on Pectin Yield

pH is considered as one of the more crucial parameters affecting the amount and properties of extracted pectin. Pectin yield increases when pH is decreased from 2.5 to 2. The highest pectin yield of 14.35% was obtained at pH 2 and lowest yield was at pH 1.5. At constant time and temperature, it was observed that the yield increased when the pH decreased from 2.5 to 2. This is likely due to the enhanced ability of acid in solubilizing the protopectin with increase in acid

strength [21]. However, further decrease in pH from 2 to 1.5 resulted in the decrease of pectin yield. Same observations were noted in conducted studies of: Miceli-Garcia [7] Gragasin [10] Liew [14] Girma [18] Methacanon [21] and Kliemann [28] wherein decrease in pH of the solution resulted in increase yield of pectin while further decrease in pH resulted in abrupt decrease in the yield.

Lower pectin yield at higher pH might be due to some pectin that might still be attached to the cell wall components, although pectin molecules can be partially hydrolyzed from plant tissues without degradation in a weak acid solution [18] [21]. On the other hand, even though a low pH is necessary to improve the yield of pectin, the strong acid solution could lead to smaller pectin particles owing to partial hydrolysis. Thus, pectin solubility would increase to the point that no precipitate is formed by the addition of alcohol [28]. The presence of high concentration of hydrogen ions in the solvent has stimulated the hydrolysis of protopectin. Protopectin is a compound formed by the combination of cellulose with pectin molecules. At low pH, as the hydrogen ion concentration of the solution is increased, ionization of the carboxylate groups is repressed, i.e., the highly hydrated carboxylate group is converted into hydrated carboxylic acid groups. The lost of carboxylate groups is able to reduce the repulsion of the polysaccharide molecules which promotes the gelation properties of pectin giving more precipitated pectin at lower pH [13].

Effect of Extraction Temperature on Pectin Yield

Pectin yield generally increases as reaction temperature increases. Highest yield was obtained at 90°C while the lowest yield was obtained at 70°C. The yield of pectin significantly increased ($p<0.05$) when the extraction temperature was increased up to 90°C, at increasing extraction time at constant pH of 2 and 2.5. High yield is attained because increasing the extraction temperature would increase the solubility of the extracted pectin, thus, giving a higher rate of extraction [18]. However, at pH 1.5, as shown in fig. 7, increasing the temperature from 70°C to 90°C, has no significant effect on the yield ($p>0.05$). It was also observed that the pectin yield decreased after the extraction temperature was increased from 80°C to 90° C, extraction time of 120 minutes and pH of 1.5. This is because, at strong acid solutions i.e. low pH, partial hydrolysis of the α-(1-4) linked units of galacturonic acid or methyl ester occur which results in pectin of lower molecular size. This low molecular weight pectin is not soluble at high temperature and will not precipitate even after the addition of alcohol [18] [28]. The result of the present study is in agreement with the results of [10], [25] and [31] wherein the pectin yield increased after the extraction temperature has been increased.

4. CONCLUSIONS

Variations in the conditions namely pH, time and temperature were investigated in this study to extract pectin from bilimbi. The yield of acid-extracted pectin range from 4.75% to 14.35% of the dry weight of the fruit. The highest yield is obtained when dried bilimbi fruit was extracted with acidified water at pH 2 for 120 minutes at 90°C. Variations in pH, temperature and time have significant effects on the yield of pectin. Indeed, this study proves that bilimbi can be a promising source of pectin.

5. RECOMMENDATIONS

Further study on the effects of different acids on pectin yield must be considered in order to determine the best medium for extraction. Moreover, optimization of pectin extraction using response surface methodology is recommended in order to fully establish the best condition for pectin extraction. Characterization of the physicochemical properties and classification of the extracted pectin is also suggested.

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